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RESEARCH ARTICLE

# Wounding Changes the Spatial Expression Pattern of the Arabidopsis Plastid ω-3 Fatty Acid Desaturase Gene (*FAD7*) through Different Signal Transduction Pathways

**Takumi Nishiuchi, Tatsurou Hamada, Hiroaki Kodama, and Koh Iba[1]**

Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812-81, Japan

**The Arabidopsis *FAD7* gene encodes a plastid ω-3 fatty acid desaturase that catalyzes the desaturation of dienoic fatty acids in membrane lipids. The mRNA levels of the Arabidopsis *FAD7* gene in rosette leaves rose rapidly after local wounding treatments. Wounding also induced the expression of the *FAD7* gene in roots. To study wound-responsive expression of the *FAD7* gene in further detail, we analyzed transgenic tobacco plants carrying the −825 Arabidopsis *FAD7* promoter–β-glucuronidase fusion gene. In unwounded transformants, *FAD7* promoter activity was restricted to the tissues whose cells contained chloroplasts. Activation of the *FAD7* promoter by local wounding treatments was more substantial in stems (29-fold) and roots (10-fold) of transgenic plants than it was in leaves (approximately two-fold). Significant induction by wounding was observed in the overall tissues of stems and included trichomes, the epidermis, cortex, vascular system, and the pith of the parenchyma. Strong promoter activity was found preferentially in the vascular tissues of wounded roots. These results indicate that wounding changes the spatial expression pattern of the *FAD7* gene. Inhibitors of the octadecanoid pathway, salicylic acid and *n*-propyl gallate, strongly suppressed the wound activation of the *FAD7* promoter in roots but not in leaves or stems. In unwounded plants, exogenously applied methyl jasmonate activated the *FAD7* promoter in roots, whereas it repressed *FAD7* promoter activity in leaves. Taken together, wound-responsive expression of the *FAD7* gene in roots is thought to be mediated via the octadecanoid pathway, whereas in leaves, jasmonate-independent wound signals may induce the activation of the *FAD7* gene. These observations indicate that wound-responsive expression of the *FAD7* gene in aerial and subterranean parts of plants is brought about by way of different signal transduction pathways.**

## INTRODUCTION

The membrane lipids of higher plants are characterized by a high proportion of polyunsaturated fatty acids. In particular, in most plant species, the fatty acids present in the galactolipids of the chloroplast membranes are ~70 to 80% trienoic fatty acids. The conversion of dienoic fatty acids to trienoic fatty acids is catalyzed by ω-3 fatty acid desaturases (Somerville and Browse, 1991). In Arabidopsis, three loci for the ω-3 fatty acid desaturases, namely, *FAD3*, *FAD7*, and *FAD8*, are responsible for the production of trienoic fatty acids (McConn and Browse, 1996). The FAD3 enzyme is localized in the microsomes, whereas the FAD7 and the FAD8 enzymes are localized in the plastid membranes (Arondel et al., 1992; Iba et al., 1993; Gibson et al., 1994). High polyunsaturated fatty acid content, including trienoic fatty acids, is considered to play an important role in the adaptation of higher plants to low, nonfreezing temperatures (Hugly and Somerville, 1992; Miquel et al., 1993; Kodama et al., 1994, 1995). An additional role for trienoic fatty acids, especially linolenic acid, has been suggested, and it is as a precursor for fatty acid–derived signaling molecules, such as jasmonate (JA) (Farmer, 1994; McConn and Browse, 1996). Linolenic acid is converted to jasmonic acid by a lipoxygenase-dependent process, namely, the octadecanoid pathway (Vick and Zimmerman, 1984; Farmer and Ryan, 1992). Several reports have suggested that linolenic acid for the octadecanoid pathway is derived not only from plasma membranes but also from plastid membranes (Vick and Zimmerman, 1984; Weiler et al., 1993; Creelman and Mullet, 1995).

The spatial expression patterns of the ω-3 desaturase genes have been investigated in several plant species. The mRNA of the *FAD3* gene was present in both the leaves and roots of Arabidopsis (Yadav et al., 1993) and tobacco (Hamada et al., 1994) but was preferentially detected in the roots of rice (Kodama et al., 1997). On the other hand, the transcript of the *FAD7* gene was observed only in photosynthetically active

[1] To whom correspondence should be addressed. E-mail kiobascb@mbox.nc.kyushu-u.ac.jp; fax 81-92-642-2621.

tissues of Arabidopsis (Nishiuchi et al., 1995) and wheat (Horiguchi et al., 1996). The −825 Arabidopsis *FAD7* promoter–β-glucuronidase (*GUS*) fusion gene conferred chloroplast-containing, tissue-specific, and light-responsive expression to transgenic tobacco plants, indicating that the expression patterns of the *FAD7* gene are transcriptionally regulated (Nishiuchi et al., 1995).

There have also been reports discussing the responses of the ω-3 desaturase genes to several environmental stresses and to a stress-related phytohormone, abscisic acid. The mRNA of the mung bean microsome ω-3 desaturase gene, which was isolated initially as an auxin-inducible gene, rapidly accumulated in hypocotyls after they were wounded (Yamamoto et al., 1992; Yamamoto, 1994). In tobacco leaves, mRNA levels of the plastid ω-3 desaturase gene also increased after wounding treatments, and this increase was accompanied by an increase in trienoic fatty acids in the major polar lipids (Hamada et al., 1996). The Arabidopsis *FAD8* gene was expressed substantially at moderately low temperatures <20°C (Gibson et al., 1994). The transcript level of the rapeseed microsome ω-3 desaturase gene was upregulated by abscisic acid treatments of microspore-derived embryos (Zou et al., 1995).

In this study, we confirmed the wound-responsive expression of the *FAD7* gene in Arabidopsis plants. We also investigated whether the wound activation of the Arabidopsis *FAD7* promoter occurs in various vegetative tissues of transgenic tobacco plants, including nonchlorophyllous tissues in which little expression of the Arabidopsis *FAD7* promoter is normally observed. In addition, we examined how jasmonic acid is involved in regulation of the wound-responsive expression of the *FAD7* promoter.

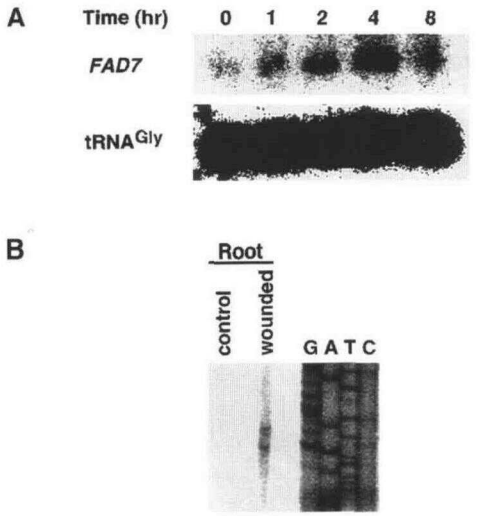


**Figure 1.** Wound-Responsive Expression of the *FAD7* Gene in Arabidopsis Plants.

**(A)** Effects of wounding treatment on mRNA levels of the Arabidopsis *FAD7* gene. Total RNAs were prepared from rosette leaves at 0, 1, 2, 4, and 8 hr after wounding. Fifteen micrograms of total RNA was loaded onto each lane. The RNA blot was probed with the Arabidopsis *FAD7* gene and then reprobed with the $tRNA^{Gly}$ gene.

**(B)** Primer extension analysis of the *FAD7* gene in wounded roots. For comparison, a sequence ladder obtained by using the same primer was run, as shown at right.

## RESULTS

### Wound-Induced Expression of the *FAD7* Gene in Arabidopsis Plants

Expression of the tobacco plastid ω-3 desaturase gene (*NtFAD7*) was previously found to be upregulated in leaves by wounding treatments (Hamada et al., 1996). Thus, we examined whether this wound-responsive expression could also be observed for the Arabidopsis *FAD7* gene. Arabidopsis rosette leaves were cut into sections with a razor blade and then soaked in sodium phosphate buffer for various lengths of time. RNA gel blot analysis showed that *FAD7* mRNA levels started to increase after 1 hr, reached a maximum after 4 hr, and began to decline 8 hr after the leaves were cut (Figure 1A). By contrast, the expression of the $tRNA^{Gly}$ gene was not affected during the course of the same experiment (Figure 1A). Similar results were obtained when Arabidopsis leaves were wounded by pressing with a mechanical hemostatic device (data not shown). Therefore, in leaves, the wound responsiveness of the plastid ω-3 desaturase gene was frequently observed in both Arabidopsis and tobacco plants.

In addition, we tested whether the wound-induced expression of the *FAD7* gene could be observed in roots of Arabidopsis plants. Roots harvested from soil-grown Arabidopsis plants were cut into sections with a razor blade and then soaked in sodium phosphate buffer in the dark. The transcripts of the *FAD7* gene were quite evident in wounded roots, and the putative transcriptional start site of the *FAD7* gene in wounded roots was identical to that reported for leaves (Nishiuchi et al., 1995), whereas no transcripts were observed in unwounded roots (Figure 1B). Thus, wounding led to the de novo expression of the *FAD7* gene in roots.

### Effects of Wounding on the Organ- and Tissue-Specific Activity of the *FAD7* Promoter

We have shown that tobacco plants transformed with a −825 Arabidopsis *FAD7* promoter–*GUS* fusion gene (designated as fD82-8 or fD82-9) exhibited higher GUS activity in leaves than in stems or roots (Nishiuchi et al., 1995). We tested whether the *FAD7* promoter could confer wound-responsive expression to leaves and other vegetative organs of these tobacco transformants. From the soil-grown

fD82-9 plants, leaf discs were made by using a cork borer, and stem slices were cut with a razor blade. The leaf discs and the stem slices were then incubated in sodium phosphate buffer under continuous illumination. In addition, roots of the same plants were sectioned with a razor blade. The root sections were then soaked in sodium phosphate buffer in the dark.

GUS activity in the leaves of the fD82-9 plant started to increase after 1 hr and reached a maximum (twofold compared with unwounded leaves) after 9 hr, after which it remained constant throughout the experimental period (Figure 2). Wounding also induced a substantial elevation of GUS activity in both stems (29-fold the activity in unwounded stems) and roots (10-fold the activity in unwounded roots) (Figure 2). The time course of the wound-induced elevation of GUS activity in stems was similar to that in leaves, whereas GUS activity in roots transiently increased 3 to 24 hr after sectioning. This wound-responsive induction in leaves, stems, and roots was also observed with two other independent transgenic lines (fD82-8 and fD82-11; data not shown). These results indicate that in tobacco plants, the wound responsiveness of the Arabidopsis *FAD7* promoter follows a pattern similar to the wound-responsive expression of the *FAD7* gene in Arabidopsis plants (Figure 1).

To visualize the wound activation of the *FAD7* promoter, we performed histochemical GUS staining with the wounded fD82-9 plants (Figure 3). In wounded stems, significant induction of GUS activity was observed in all tissues, including those of the trichomes, epidermis, cortex, vascular system, and pith of the parenchyma (Figures 3A, 3B, 3E, and 3F). The strongest histochemical staining was observed in the vascular system of the stem. In petioles, the wound-mediated modulation of *FAD7* promoter–*GUS* expression was very similar to that observed in stems (Figures 3C and 3D). In wounded roots, GUS activity was detected mainly in the vascular tissues (Figures 3I and 3J). These histochemical experiments demonstrate that wounding changed the tissue-specific expression of the *FAD7* gene.

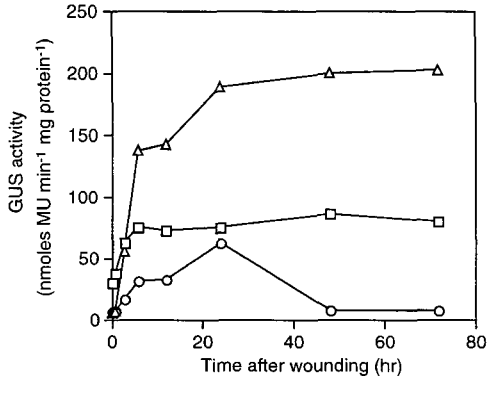


**Figure 2.** Time Course of Wound Activation of the *FAD7* Promoter–*GUS* Fusion Gene in Young Vegetative Organs of Transgenic Tobacco Plants.

Portions of young leaves (squares), stems (triangles), and roots (circles) of mature fD82-9 plants were harvested at various times after wounding, and GUS activities in their tissues were determined. Each value represents the average of two independent samples. MU, 4-methylumbelliferone.

## Effects of Light and of Developmental Factors on Wound Induction of *FAD7* Promoter Activity

The expression of the Arabidopsis *FAD7* gene in unwounded leaves required light (Nishiuchi et al., 1995). Thus, it is possible that light affects the wound-responsive expression of the *FAD7* gene. The vegetative organs of fD82-9 plants were cut into pieces, and the pieces were incubated in sodium phosphate buffer under light or in the dark for 6 hr. In leaves and stems, wounding treatments caused an increase in GUS activity, regardless of light conditions (Figure 4A). The rates of induction of GUS activity resulting from wounding were higher under light than were those observed in the dark, indicating that light exerts positive effects on the wound activation of the *FAD7* promoter in these organs (Figure 4A). By contrast, light attenuated the wound-inducing activity of the *FAD7* promoter in roots (Figure 4A), although in unwounded roots, illumination did not affect the expression of the *FAD7* gene (data not shown).

In addition, we tested whether the wound responsiveness of the *FAD7* promoter is the same in different portions of each vegetative organ. Leaves, stems, and roots in three parts of the plants, as shown in Figure 4C, were wounded. In leaves and stems, the rates of induction of GUS activity resulting from wounding were much higher in the upper parts of plants than those in the lower parts (Figure 4B). The parts containing wounded root tips exhibited the highest induction of *FAD7* promoter activity compared with other older parts of the roots (Figure 4B). These results indicate that high wound responsiveness of the *FAD7* promoter was frequently observed in young tissues of all of the vegetative organs examined in this study.

## Effects of Inhibitors of the Octadecanoid Pathway on the Wound-Responsive Expression of the *FAD7* Promoter

It has been proposed that the activation of several defensive genes by wounding occurs via the octadecanoid pathway. Here, linolenic acid is converted to jasmonic acid, leading to the transcriptional activation of defensive genes (Vick and Zimmerman, 1984; Farmer and Ryan, 1992). To examine whether wound-responsive expression of the *FAD7* gene is caused via the octadecanoid pathway, the influence of two effective inhibitors of the octadecanoid pathway, salicylic acid (SA) and *n*-propyl gallate (*n*-PG), on *FAD7* expression was investigated using fD82-8 plants.

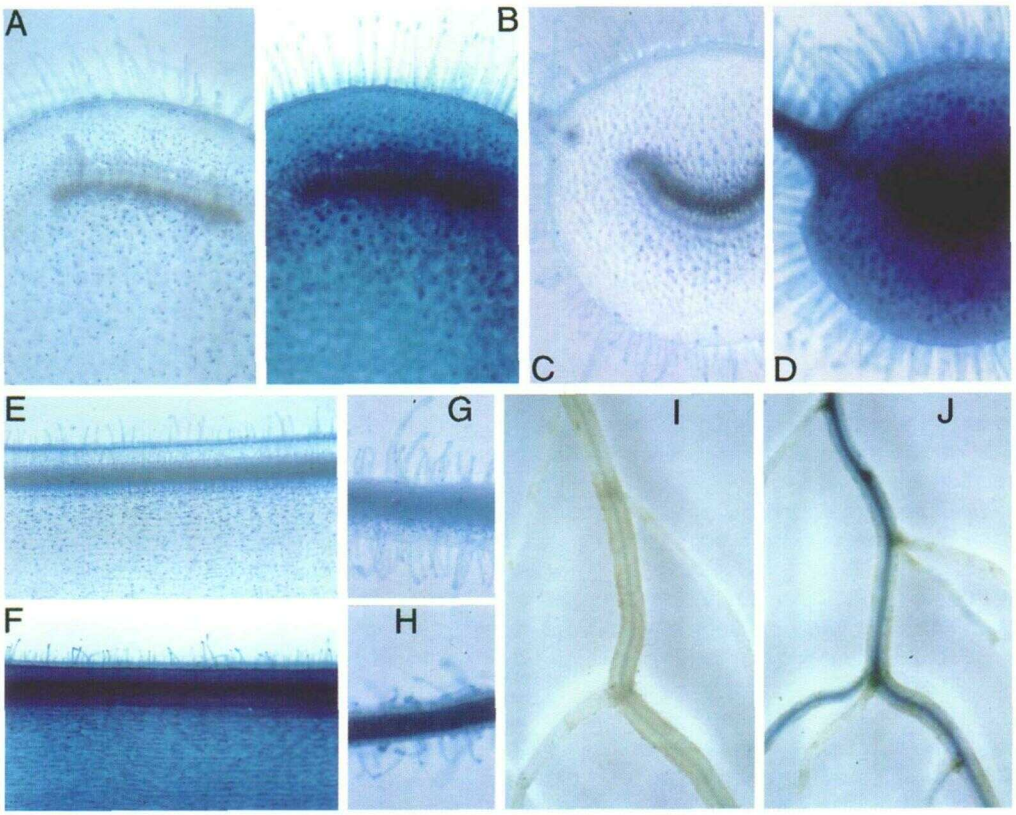


**Figure 3.** Histochemical GUS Staining of Wounded and Unwounded Vegetative Tissues of the *FAD7* Promoter–*GUS* Transformants (fD82-8 Line).

**(A)** and **(B)** Transverse sections of unwounded and wounded young stems, respectively.
**(C)** and **(D)** Cross-sections of unwounded and wounded young petioles, respectively.
**(E)** and **(F)** Longitudinal sections of unwounded and wounded young stems, respectively.
**(G)** and **(H)** Cross-sections of unwounded and wounded young leaves, respectively.
**(I)** and **(J)** Segments of unwounded and wounded roots, respectively.

It is well known that in tomato plants, SA effectively blocks the octadecanoid pathway (Peña-Cortés et al., 1993; Doares et al., 1995a). *n*-PG strongly inhibits the activity of the tobacco lipoxygenase that is involved in JA biosynthesis (Fournier et al., 1993; Ellard-Ivey and Douglas, 1996). Therefore, local wounding treatments were performed using detached leaves that had been fed with inhibitor solutions or distilled water via the petiole (Figure 5A). Wound induction of *FAD7* promoter activity in SA- or *n*-PG–pretreated leaves was the same as that seen in leaves fed with distilled water, indicating that prefeedings with these inhibitors did not inhibit wound activation of the *FAD7* promoter in leaves (Figure 5A). Stems of the fD82-8 plants were fed with inhibitor solutions or distilled water through their basal portions. Local wounding treatments were then performed on the upper portions of the plants (Figure 5B). After prefeeding with inhibitors or distilled water, GUS activity in the upper portion of the stems (zero time) increased (Figure 5B). This increase was probably due to the systemic wounding that was caused when shoots were cut from the basal portion of the stems with a razor blade. Subsequent local wounding treatments led to significant increases in GUS activity in the upper portion of stems, regardless of prefeedings with SA or *n*-PG (Figure 5B). These results indicate that neither SA nor *n*-PG inhibited the wound activation of the *FAD7* promoter in stems. In addition, the fD82-8 plants were hydroponically fed with inhibitors or distilled water, and their roots were then wounded (Figure 5C). In contrast to leaves and stems, both SA and *n*-PG caused severe inhibition of wound-induced elevation of GUS activity in roots (Figure 5C).

### Effects of Methyl JA on the *FAD7* Promoter Activity

To evaluate whether jasmonic acid is involved in the regulation of *FAD7* gene expression, we sprayed the leaves on the

upper parts of fD82-8 plants (defined in Figure 4C) with a methyl JA (MeJA) solution, and we determined GUS activity in leaves, stems, and roots. Three hours after the spraying with MeJA, GUS activity in the directly sprayed leaves declined to ~30% of that seen in the corresponding leaves of the untreated plants and then remained constant for at least the next 3 hr (Figure 6A). In roots, GUS activity began to increase within the first 3 hr, reaching a level approximately threefold of that seen in roots of untreated plants 6 hr after being sprayed. Thus, MeJA exerts negative effects on *FAD7* promoter activity in leaves, whereas it activates the *FAD7* promoter in roots.

Exposure of the fD82-8 plants to MeJA vapor caused similar effects on *FAD7* promoter activity, and a slight but significant increase in promoter activity was observed in roots (Figure 6B), suggesting that besides the MeJA-derived signals that were transported from the leaves to roots, MeJA itself mediates the activation of the *FAD7* promoter in roots. In addition, hydroponic feeding of a MeJA solution activated drastically the *FAD7* promoter in roots (Figure 6B). This activation of the *FAD7* promoter in roots occurred in a dose-dependent manner when the concentration of MeJA was within the range of 1 to 100 μM (Figure 6C). Simultaneous application of SA with the MeJA solution effectively inhibited MeJA-responsive elevation of GUS activity (Figure 6C).

### Wound- and MeJA-Responsive Expression of the Endogeneous *FAD7* Gene in Tobacco Plants

To determine whether the behavior of the endogeneous tobacco *FAD7* gene (*NtFAD7*) mimics that of the transgene,

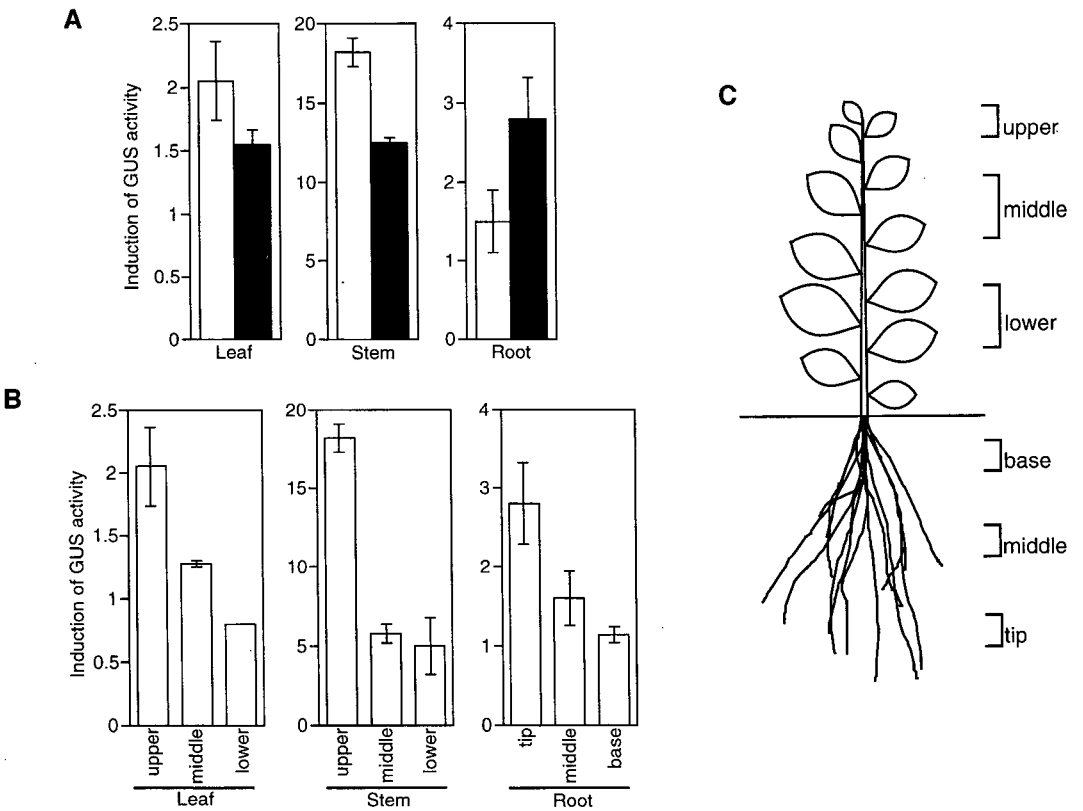


**Figure 4.** Light and Developmental Factors Affecting Wound Activation of the *FAD7* Promoter in Each Vegetative Organ.

**(A)** Effects of light on the wound-responsive expression of the *FAD7* promoter. GUS activity was determined in young vegetative organs of fD82-9 plants that had been wounded and then left for 6 hr under light (open bars) or in the dark (closed bars). The ratios of the GUS activity of wounded tissue to the GUS activity of the corresponding unwounded tissue are compared. All values are means ±SD ($n = 5$).

**(B)** The wound induction of the *FAD7* promoter in different portions of the vegetative organs of fD82-9 plants. Each vegetative organ was divided into three portions, as shown in **(C)**, and these were wounded as described in Methods. The leaf and stem sections were incubated under light for 6 hr; root sections were incubated in the dark for 6 hr. Induction of GUS activity by wounding was determined as given in **(A)**. All values are means ±SD ($n = 5$).

**(C)** Sections used for the experiment in **(B)**.

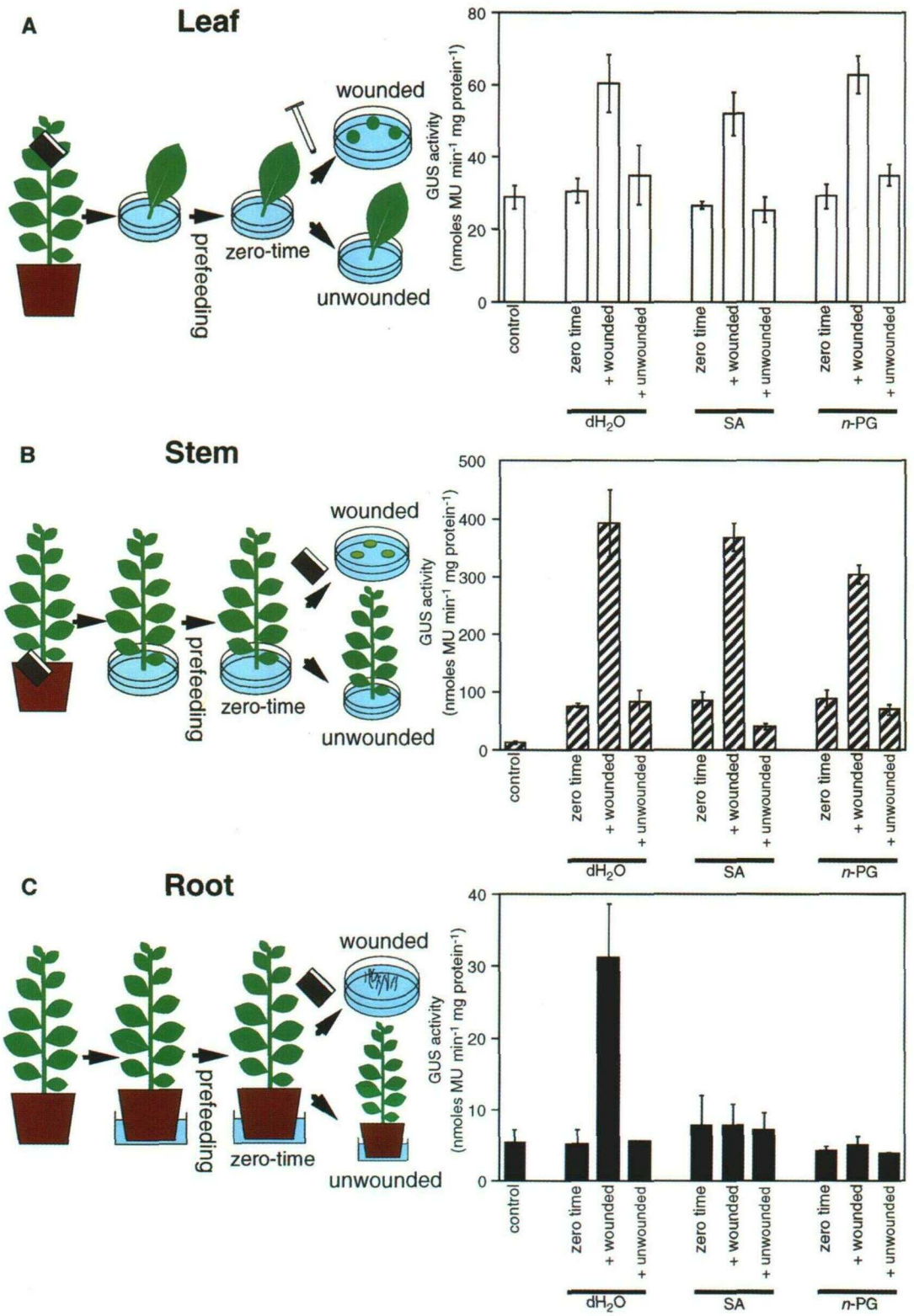


**Figure 5.** Effects of Inhibitors of the Octadecanoid Pathways on the Wound Induction of *FAD7* Promoter Activities in Leaves, Stems, and Roots of fD82-8 Plants.

we analyzed how wounding of stems and roots and applying MeJA to leaves and roots affected *NtFAD7* expression. Figure 7 shows that wounding clearly induced expression of *NtFAD7* in stems and roots. In addition, the exogeneous application of MeJA induced expression of *NtFAD7* in roots but repressed its expression in leaves. These data suggest that the endogeneous *FAD7* gene shows an expression pattern similar to that observed for the transgene (Figures 2 and 6).

## DISCUSSION

### Wounding Modulates Organ- and Tissue-Specific Expression of the *FAD7* Gene

Histochemical GUS staining of the *FAD7* promoter–*GUS* transformants showed that in unwounded plants, *FAD7* promoter activity was localized within tissues containing chloroplasts in both leaves and stems but not in any regions of the roots (Nishiuchi et al., 1995). However, wounding markedly modified the tissue-specific expression pattern of the *FAD7* gene in vegetative organs (Figure 3). Wounding caused not only an enhancement of the expression of the *FAD7* gene in tissues containing chloroplasts, for example, mesophyll tissues of leaves (Figures 3G and 3H), but also significant induction of *FAD7* expression in nonchlorophyllous tissues. In particular, strong GUS activities were observed in all types of cells in both wounded stems and petioles (Figures 3B, 3D, and 3F). It seems that the effects of wounding on *FAD7* gene expression in nonchlorophyllous tissues are much greater than they are in chlorophyll-containing tissues. In fact, the absolute activity of the *FAD7* promoter in stems was approximately double that seen in leaves 24 hr after wounding (Figure 2).

It has been reported that the spatial expression patterns of several defensive genes, such as genes for chitinase, proteinase inhibitors, and ascorbate free radical reductase, are modulated by wounding (Chang et al., 1995; Grantz et al., 1995; McGurl et al., 1995; Xu et al., 1996). In unwounded plants, these defensive genes were constitutively expressed at high levels in roots but at considerably lower levels in leaves. Wounding, however, not only enhanced the expression of these genes in roots but also induced their expression in leaves. These observations imply that modulation of organ-specific expression patterns of these wound-inducible genes can be placed into two categories, one being an enhancement of expression in tissues in which the genes are constitutively expressed in unwounded states and the other being de novo induction of expression in tissues in which the genes are not expressed under unwounded conditions.

Does modulation of gene expression in response to wounding occur via the same wound signals in all organs? The signal transduction pathways of wound-induced gene expression in each of the organs within the same plant species have not been studied systematically. Although the highest level of wound induction of *FAD7* promoter activity was observed in the young portions of all of the vegetative organs examined here (Figure 4), the effects of light, MeJA application, and pretreatments with inhibitors of the octadecanoid pathway on wound responsiveness in roots were completely different from those seen in leaves and stems (Figures 4 to 6). These results indicate that wound-responsive expression of the *FAD7* gene in the aerial and subterranean parts of plants is brought about via different signal transduction pathways.

### Wound Activation of the *FAD7* Promoter in Roots Occurs via the Octadecanoid Pathway

In higher plants, jasmonic acid and MeJA are naturally occurring regulators of a plant's development and response to external stimuli, including wounding (Sembdner and Parthier, 1993; Creelman and Mullet, 1995). Wounding causes rapid accumulation of jasmonic acid in leaves and stems by way of the octadecanoid pathway (Creelman et al., 1992; Seo et al., 1995; Conconi et al., 1996). The wound-induced accumulation of jasmonic acid modulates the expression of several defensive genes, such as the proteinase inhibitor II (*pin2*) gene (Creelman et al., 1992; Farmer and Ryan, 1992; Peña-Cortés et al., 1993). The *FAD7* promoter was significantly activated in roots by wounding and also by the application of MeJA (Figures 2, 3, and 6). In addition, both SA and *n*-PG strongly inhibited the wound responsiveness of the *FAD7* promoter in roots (Figures 5C and 6C). In addition,

**Figure 5.** (continued).

**(A)** Promoter activity in leaves.
**(B)** Promoter activity in stems.
**(C)** Promoter activity in roots.
GUS activities in each organ of healthy fD82-8 plants were assayed as a control. Prefeeding of leaves, stems, and roots with inhibitor solutions or distilled water ($dH_2O$) was performed as illustrated. GUS activities of aliquot samples of each organ were then determined as "zero time." Other aliquot samples of each organ fed with the inhibitors or distilled water were cut into leaf discs, stem slices, or root segments. These were incubated in sodium phosphate buffer for 6 hr. GUS activities were then assayed as "+ wounded." Alternatively, samples were directly transferred to the sodium phosphate buffer and incubated for 6 hr. GUS activities were then assayed as "+ unwounded." Values are means ±SD ($n$ = 5). MU, 4-methylumbelliferone.

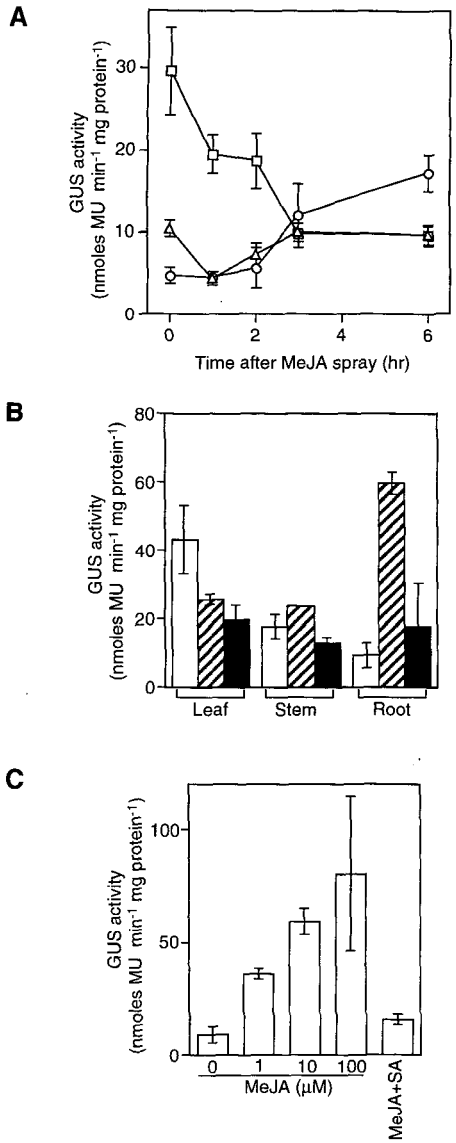


**Figure 6.** Effects of MeJA Application on the Expression of the *FAD7* Promoter in Vegetative Organs of fD82-8 Plants.

**(A)** Responses of the *FAD7* promoter in MeJA-sprayed plants. Upper leaves of intact fD82-8 plants were sprayed with a 100 μM MeJA solution. GUS activities were determined in leaves (squares), stems (triangles), and roots (circles).

**(B)** GUS activities in fD82-8 plants exposed to MeJA, using two different methods of application. Using the vapor method (closed bars), entire plants were placed in air-tight glass chambers containing MeJA vapor for 6 hr. For hydroponic feeding (hatched bars), whole plants together with their pots were dipped in a 10 μM MeJA solution for 6 hr. GUS activities were also determined in each organ of unwounded plants that did not receive MeJA treatment as controls (open bars).

**(C)** Dose response of roots to the induction of the *FAD7* promoter by MeJA. Intact plants were hydroponically fed with MeJA solutions of different concentrations for 6 hr. The results of simultaneously applying 1 mM SA together with 10 μM MeJA on the expression of the *FAD7* promoter in roots are shown. GUS activities were determined in the roots of these plants.

All results are given as mean ±SD ($n$ = 5). MU, 4-methylumbelliferone.

SA also inhibited the MeJA activation of the *FAD7* promoter in roots (Figure 6C). These observations suggest that the properties of the wound activation of the *FAD7* promoter in roots are similar to those of the *pin2* gene (Peña-Cortés et al., 1993; Doares et al., 1995a).

It has not been reported whether endogenous jasmonic acid levels in roots are increased by wounding. However, jasmonic acid has been clearly detected in the roots of soybean seedlings (Creelman and Mullet, 1995). In particular, the root tips contained high levels of jasmonic acid in comparison to hypocotyls and leaves, indicating that jasmonic acid biosynthesis actually occurs within the roots of higher plants (Creelman and Mullet, 1995). In addition, MeJA led to a significant increase in the mRNA levels of the Arabidopsis lipoxygenase gene (*LOX1*; Bell and Mullet, 1993; Melan et al., 1993) and vegetative storage protein genes (*vspA* and *vspB*; Berger et al., 1995) in roots. Thus, the mechanism involved in the transcriptional activation by JA is considered to be functional in roots. Taken together, the wound-induced expression of the *FAD7* gene in roots would occur via the octadecanoid pathway, as has been observed already in wound-responsive genes, such as the *pin2* gene in leaves (Doares et al., 1995b).

A G-box motif has been shown to constitute the MeJA-responsive domain in the promoter regions of both the soybean *vspB* gene (Mason et al., 1993) and the potato *pin2* gene (Kim et al., 1992). The G-box motif can be found upstream of the many stress-related genes, including the wound- and JA-responsive genes (Williams et al., 1992; Daugherty et al., 1994). A G-box–like motif has also been found in the promoter region of the *FAD7* gene (Nishiuchi et al., 1995), suggesting the possibility that this sequence is involved in the MeJA responsiveness of the *FAD7* promoter in roots.

### Wound Activation of the *FAD7* Promoter Does Not Require Any Jasmonic Acid Biosynthesis in Leaves

In leaves, the *FAD7* promoter was upregulated by wounding treatment (Figure 2) but not by the application of MeJA (Figure 6). In addition, prefeedings of both SA and *n*-PG exerted no inhibitory effect on wound-induced *FAD7* gene expression in leaves (Figure 5). These results suggest that in leaves, wound activation of the *FAD7* promoter did not occur by way of the octadecanoid pathway. Although the mechanisms involved in the wound-responsive expression of the *FAD7* gene in leaves are as yet barely understood, several factors could be considered candidates for mediating wound signals. Systemin and cell wall fragments, which

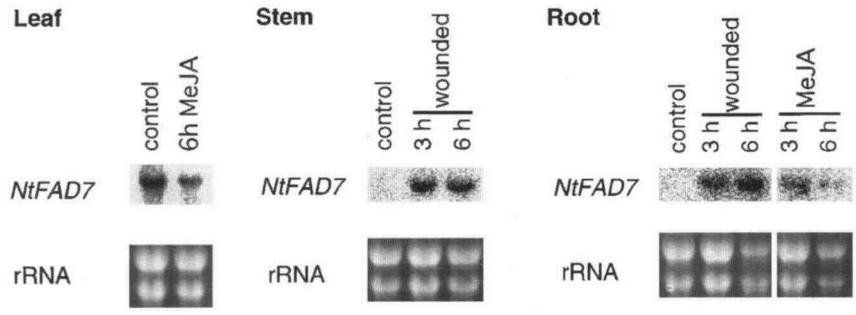


**Figure 7.** Wound- and MeJA-Responsive Expression of the Endogenous Tobacco *FAD7* Gene.

Wounding of stems and roots of wild-type tobacco plants was performed as described in Methods. The application of MeJA to leaves and roots was performed by spraying and hydroponic feeding, respectively. Total RNA was prepared from leaves, stems, and roots as indicated at the times (hours [h]) after wounding or MeJA application. Fifteen micrograms of total RNA was loaded onto each lane. Blots were probed with the *NtFAD7* gene. The equivalence of RNA loading among lanes of agarose gels was demonstrated by ethidium bromide staining of rRNA bands.

are considered to be the primary signaling molecules for the octadecanoid pathway (Doares et al., 1995b), may modulate the wound responsiveness of the *FAD7* promoter in leaves by direct interaction or through novel pathways distinct from those of the octadecanoid pathway. An alternate possibility is the regulation of the induction of the *FAD7* gene by protein kinases, which recently have been reported to be rapidly and transiently activated by wounding treatments in tobacco leaves (Seo et al., 1995; Usami et al., 1995).

In contrast to most wound-responsive genes, genes encoding chloroplast-localized proteins, such as the small and large subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the light-harvesting chlorophyll *a/b* binding proteins (LHCPII), are negatively regulated by MeJA in leaves (reviewed in Reinbothe et al., 1994). These effects of MeJA ultimately lead to symptoms of senescence within plastid compartments as a result of chlorophyll loss and degradation of Rubisco and LHCPII (Weidhase et al., 1987). MeJA caused a significant decrease in *FAD7* promoter activity in unwounded leaves (Figures 6A and 6B), suggesting the possibility that jasmonic acid possesses the potential to repress a broad range of genes involved in the establishment of chloroplast functions.

In this study, we did not examine the physiological roles played by the *FAD7* gene in the wound response of higher plants. The relationship between the expression of the *FAD7* gene and the regulation of jasmonic acid and linolenic acid levels with regard to wound response of higher plants needs to be investigated.

## METHODS

### Plant Materials and Growth Conditions

Plants (*Arabidopsis thaliana* ecotype Columbia) were grown in soil at 25°C under continuous fluorescent illumination (2000 lux). Tobacco (*Nicotiana tabacum* cv W38) plants harboring the −825 Arabidopsis *FAD7* promoter–β-glucuronidase (*GUS*) fusion gene were generated as previously described (Nishiuchi et al., 1995). Three independently transformed lines—fD82-8, fD82-9, and fD82-11—were selected as representatives of *FAD7* promoter-driven expression. Kanamycin-resistant $R_1$ transformants of these lines were grown in soil at 25°C under continuous light illumination (2000 lux) for 3 months.

### Wounding Treatments

The rosette leaves and roots of 4-week-old Arabidopsis plants were cut into ~1-cm-long sections with a sterile razor blade and then soaked in 50 mM sodium phosphate buffer, pH 7.0. Sections of the leaves and roots were incubated under continuous light (2000 lux) and in the dark, respectively.

The wounding treatments of tobacco plants were performed as follows. Leaves were cut into discs (7.5 mm in diameter) with a cork borer. The discs were immediately floated on 50 mM sodium phosphate buffer, pH 7.0, under the light. Stems were cut into ~1-mm-thick slices with a razor blade, and the slices were then incubated in sodium phosphate buffer under the light. Roots were washed briefly to remove any soil, cut into ~1-cm-long sections with a razor blade, and subsequently soaked in sodium phosphate buffer with no illumination. After wounding treatments, fluorometric and histochemical GUS assays were performed.

### Prefeeding with Inhibitor Solutions

The prefeeding of leaves, stems, and roots of the fD82 plants with inhibitor solutions (salicylic acid [SA] or *n*-propyl gallate [*n*-PG]) or distilled water was performed as follows. Leaves were detached from the plants by cutting the petiole portion with a razor blade. The cut ends were then incubated in 1 mM SA solution for 3 hr, in 50 μM *n*-PG solution for 14 hr, or in distilled water for 3 hr. The stem prefeeding method involved cutting the basal portion of the stem with a razor blade to obtain shoots. The shoots were then arranged vertically in a small vial filled with 1 mM SA solution, 50 μM *n*-PG solution, or distilled water and allowed to stand for 5, 14, or 5 hr, respectively.

Roots were prefed by placing entire plants together with their pots into plastic bowls containing 1 mM SA solution for 5 hr, 50 μM *n*-PG solution for 14 hr, or distilled water for 5 hr.

### Methyl Jasmonate Application

Methyl jasmonate (MeJA) was applied to tobacco plants under continuous light (2000 lux) by one of the following three methods. (1) A solution of 0.5 mL of 100 μM MeJA was sprayed onto the upper leaves of unwounded plants with a commercial atomizer. GUS activities were fluorometrically determined in each organ at 0, 1, 2, 3, and 6 hr after the spraying. (2) Plants were transferred to air-tight glass chambers, and a cotton ball that had been dampened with 1 mL of 100 μM MeJA solution was placed at the bottom of the chambers (the vapor method). The plants were incubated for 6 hr. (3) Soil-grown plants together with their pots were hydroponically fed by dipping them into the MeJA solution (1 to 100 μM) and allowing them to stand for 6 hr. After the MeJA treatment, GUS activities in leaves, stems, and roots were fluorometrically determined.

### RNA Gel Blot Analysis

Total RNA was extracted as described by Puissant and Houdebine (1990) from Arabidopsis and tobacco plants. RNA gel blot analysis was performed as described previously using the following hybridization probes: the $^{32}$P-labeled 3′ noncoding region of the Arabidopsis *FAD7* cDNA (Iba et al., 1993), the rice $tRNA^{Gly}$ gene (Reddy and Padayatty,1988), and the 1.4-kb *NtFAD7* cDNA (Hamada et al., 1996).

### Primer Extension Analysis

An 18-bp oligonucleotide (dpe1; 5′-dTGTGAGAGAAACTTGTGT-3′) was labeled at the 5′ end with $\gamma$-$^{32}$P-ATP by using T4 polynucleotide kinase. The labeled oligonucleotide was annealed to 50 μg of total RNA isolated from each sample. The polymerization reaction was conducted with avian myeloblastosis virus reverse transcriptase (Life Science, Inc., St. Petersburg, FL), as described by Sambrook et al. (1989). The products of the primer extension reaction were electrophoresed on a polyacrylamide gel.

### Fluorometric and Histochemical GUS Assays

Fluorometric and histochemical GUS assays were conducted essentially as described previously (Nishiuchi et al., 1995). Histochemical GUS staining of unwounded and wounded sections was performed as follows. Each tissue was cut off and then immediately incubated in 1 mM 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-gluc) solution as an "unwounded" section, or each cut section was incubated in sodium phosphate buffer for 6 hr and then stained in 1 mM X-gluc solution as a "wounded" section.

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